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Polyploidisation of *Lobelia speciosa* and Introgression in *Lobelia erinus* Hybrids

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Summary

Lobelia erinus hybrids are important bedding plants worldwide. Despite their significance, information about their breeding history is scarce. An interspecific background can be assumed, however, only a few reports about interspecific hybridisation of *Lobelia erinus* hybrids are available. So far, *Lobelia* plants up to the hexaploid level have been known.

This work reports for the first time on an octoploid *Lobelia* (2n=8x=56) which was obtained by mutagenesis of a diploid *Lobelia speciosa*. Furthermore, this paper demonstrates that cross-combination of the octoploid mutant with tetraploid *Lobelia erinus* hybrids results in hexaploid plants.

Key words. *Campanulaceae* – *Lobelia* – ploidy – interspecific hybridisation

Introduction

Among about 365 species of the genus *Lobelia* (Wimmer 1953; Zander 2002) there are several species of ornamental interest. *Lobelia erinus* L. belongs to a genetic diversity centre in South Africa (Lammers 1993). It it is considered as the main genetic background for the economically very successful *Lobelia erinus* hybrids. Their ornamental value is appreciated since the 17th century. Breeding efforts resulted in cultivars with colour shades of white, pink, lavender, purple and blue. Despite the long and intense breeding history and although cultivars of *Lobelia erinus* hybrids are very important bedding plants, there is only little known about their breeding history. Interspecific hybridisation as one of the breeding methods seems to be very likely (Horn 1996). Consequently, the introgression of wild species is a proper method to widen the genetic background. Current cultivars of *Lobelia erinus* hybrids are characterised by deficiencies in heat-, cold-, and shade-tolerance and they often do not flower continuously till the frost. In contrast, *Lobelia speciosa* fulfils demands like continued flowering both in direct sun and under shady conditions. It recovers easily due to its strong growth also after bad weather periods and it continues flowering till the frost. Therefore, cross-breeding of *Lobelia spec.* with cultivars of *Lobelia erinus* hybrids is the goal of this evaluation.

Systematic studies about the crossability of *Lobelia* species are not known. Fortunately, numerous studies on chromosome numbers provide an overview about the ploidy levels in the genus *Lobelia*. All investigated species are based on a polyploid series with x=7. Diploid (2n=2x=14), tetraploid (2n=4x=28), and hexaploid (2n=6x=42) *Lobelia* species are known (Thulin 1970, 1983; Lammers and Hensold 1992; Lammers 1993; Viera and Shepherd 1996; Ruas et al. 2001). According to these literature reports *Lobelia erinus* is diploid. In addition, tetraploid accessions were found in nature. Thus all described hexaploid forms in *Lobelia erinus* are the result of breeding (Armand 1912; De Vilmorin and Simonet 1927; Sugiura 1936; Okuno 1937; Thulin 1983).

Materials and Methods

Plant Material

This study is based on the following cultivars of *Lobelia erinus* hybrids: 'Hot White', 'White Star', 'Big Blue' and a wild accession of *Lobelia spec.*, which was identified later as *Lobelia cuneifolia* Link & Otto (private communication with Christopher Cupido, Kirstenbosch Research Centre, RSA). This taxonomical description should be used with caution because of some controversy between formerly published chromosome counts of this species (Lammers and Hensold 1992; Lammers 1993) and the results of the present work as it will be discussed below.

Colchicine treatment, chimeral segregation and ploidy analysis

Lobelia spec. was cultivated in Dresden, in the greenhouse (22 °C) using pots with 12 cm in diameter. All branches were pinched down to 5 cm. After two days, about 50 axillary buds of *Lobelia spec.* were treated with colchicine (0.2 % concentration) during the morning hours in order to double the chromosome set (Satina et al. 1940; Hansen and Andersen 1996; Olbricht et al. 2006; Ojiewo et al.

2007). This procedure was repeated twice. Stomata lengths were measured as an indication for the ploidy of the first apex layer (Satina et al. 1940; Schwanitz 1952; Olbricht et al. 2006). After suchlike mutagen treatments, a chimeral constitution of each plant has to be anticipated. These mutations cause changes in the morphology of the plants like enlarged or crippled leaves, larger flowers and compact growth (Thao et al. 2003; Olbricht et al. 2006; Jadrná et al. 2010). Somatic segregation of a putative chimera can be obtained after *in vitro* callus culture (Cassells and Minas 1983; Tilney-Bassett 1986; Plaschil et al. 2003, Olbricht et al. 2006, Plaschil and Olbricht 2008). In consequence, homohistonts can be formed. Leaf explants of the putative chimera were regenerated into flowering plants. A modified MS-medium enriched with phytohormones was used (Murashige and Skoog 1962).

Chromosome counting was done for the original accession *Lobelia spec.*, for a *Lobelia spec.* mutant (henceforth named as *Lobelia spec.* polyploid) regenerated via callus, for the cultivar of *Lobelia erinus* hybrid 'Hot White' and for the hybrids from cross-combination of the *Lobelia spec.* polyploid with the cultivars 'Hot White' and 'Big Blue'. Root tips were fixed in hydro-oxychinolin (0.002 M) for four hours and were transferred into a Carnoy solution afterwards. Alcoholic hydrochloric acid-carmine was used for staining of squash preparations (Snow 1963). One hundred stomata were measured for each variant using a light microscope Olympus BX 50. Likewise, chromosomes were counted considering ten cells per variant.

Cross-combination

Cross-combination was performed using the following combinations:

1. *Lobelia spec.* polyploid × *Lobelia erinus* hybrid 'Hot White'
2. *Lobelia erinus* hybrid 'Big Blue' × *Lobelia spec.* polyploid.

Female flowers were pollinated by hand and were covered with little bags to prevent undesired cross-pollination.

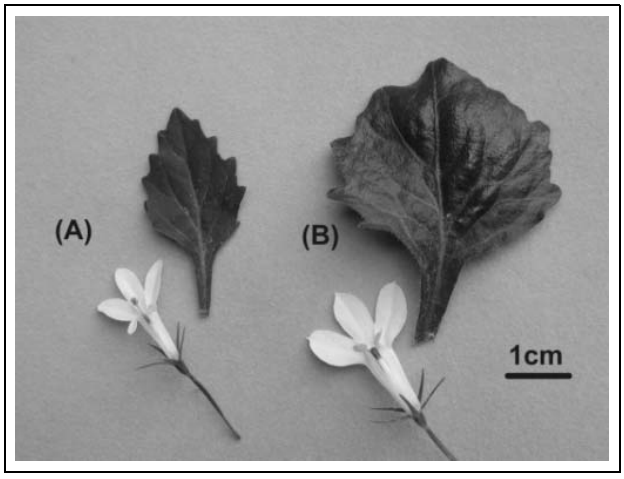


Fig. 1. Leaves and flowers of the native form *Lobelia spec.* (A) and of a large flowering mutant after colchicine treatment and *in vitro* segregation (B).

Results

Polyploidisation, in vitro callus culture and ploidy analysis

After colchicine treatment of axillary buds of *Lobelia spec.* a mutant branch with considerable large flowers and broad leaves was selected and maintained as a clone by vegetative propagation. The clone plants showed a compact growth habit in comparison to the trailing character of the *Lobelia spec. In vitro* callus culture of this large flowering mutant resulted in plants with small flowers similar in size to the original *Lobelia spec.* and in large flowering types. One of the large flowering types was selected as *Lobelia spec.* polyploid (Fig. 1). The stomata lengths of *Lobelia spec.* and *Lobelia spec.* polyploid were measured as 39.8±4.3 µm and 59.6±4.2 µm, respectively (Figs. 2A and 2B). Thus, a stomata length ratio of 1 to 1.5. was found. Chromosome counting revealed 14 chromosomes for *Lobelia spec.* (Fig. 3A) and 56 for *Lobelia spec.* polyploid (Fig. 3B). Consequently, *Lobelia spec.* can be considered as diploid with (2n=2x=14) and *Lobelia spec.* polyploid can be considered to be octoploid (2n=8x=56). For comparison and in regard to cross-combination experiments the *Lobelia erinus* hybrid 'Hot White' was characterised as tetraploid with (2n=4x=28; Fig. 3C). The stomata length measurement of the *Lobelia erinus* hybrid 'Big Blue' amounts to 46.2±3.7 µm which suggests a tetraploid state, too.

Cross-combination

The *Lobelia spec.* polyploid was proved fully fertile. After selfing, it resulted in similar seedlings with only little visible variation in plant morphology. It can be considered as auto-polyploid (auto-octoploid). F1 seedlings of the cross-combination *Lobelia spec.* polyploid × *Lobelia erinus* hybrid 'Hot White' (No. 1) resulted in types with white and bluish flowers which are larger than those of the parent plants (Fig. 4). As the result of the cross-combination *Lobelia erinus* hybrid 'Big Blue' × *Lobelia spec.* polyploid (No. 2), types with intermediate and larger flowers were obtained. With regard to flower colour, only intermediate types were found (Fig. 5). The two seedling selections named "Lob 7" from combination No. 1 and "Lob 5" from combination No. 2 both revealed a number

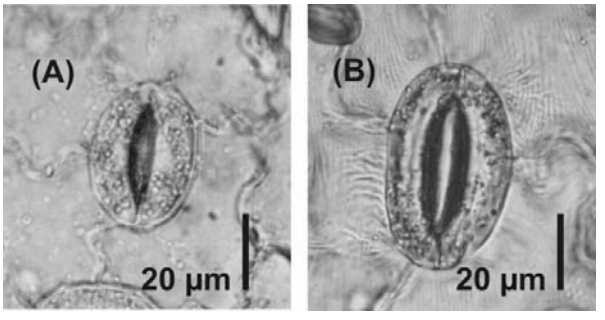


Fig. 2. Stomata of *Lobelia spec.* (A) and of a large flowering mutant after colchicine treatment and *in vitro* segregation (B).

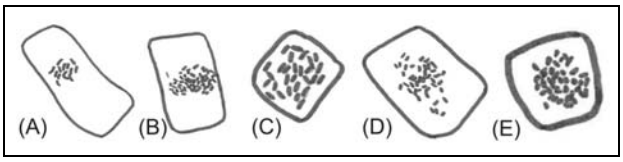


Fig. 3. Images of chromosomes showing on average 2n=2x=14 for *Lobelia spec.* (A), 2n=8x=56 for *Lobelia spec.* polyploid (B), 2n=4x=28 for the *Lobelia erinus* hybrid 'Hot White' (C), and 2n=6x=42 for the seedling selections "Lob 7" (D) and "Lob 5" (E).

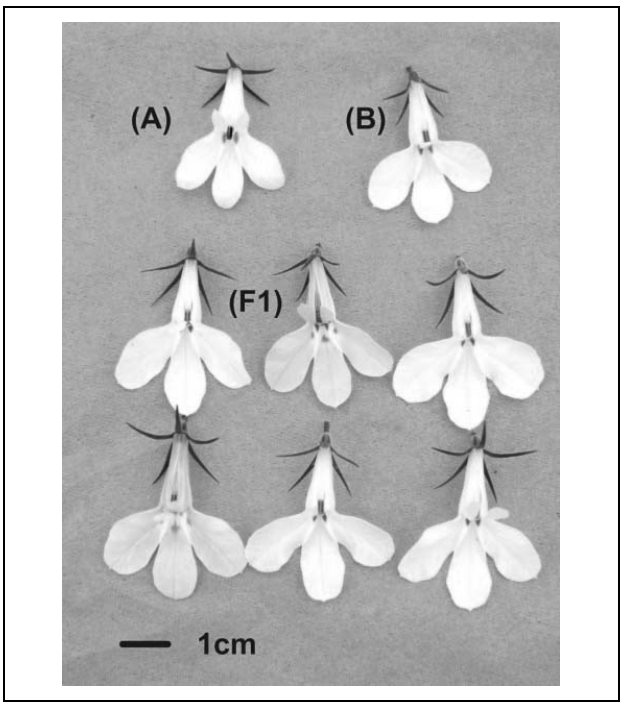


Fig. 4. Flowers of F1 seedlings of cross-combination No. 1, *Lobelia spec.* polyploid (A) × *Lobelia erinus* hybrid 'Hot White' (B).

of 42 chromosomes. Consequently, they are hexaploid plants (2n=6x=42; Figs. 3D and 3E).

Discussion

The presented work is the first report about an octoploid *Lobelia* with 2n=8x=56. The plant was obtained after colchicine treatment of axillary buds of a diploid *Lobelia spec.* and subsequent *in vitro* callus culture. Callus regenerated plants from leaf explants of a large flowering mutated branch showed either small or large flowers. This and the somatic segregation after *in vitro* callus culture indicate the chimeral character of the mutant branch (Olbricht et al. 2006; Rubuluza et al. 2007, Nilanthi et al. 2009).

As mentioned above *Lobelia spec.* was identified as *Lobelia cuneifolia* Link & Otto (private communication with Christopher Cupido, Kirstenbosch Research Centre, RSA). According to the literature, *Lobelia cuneifolia* is a

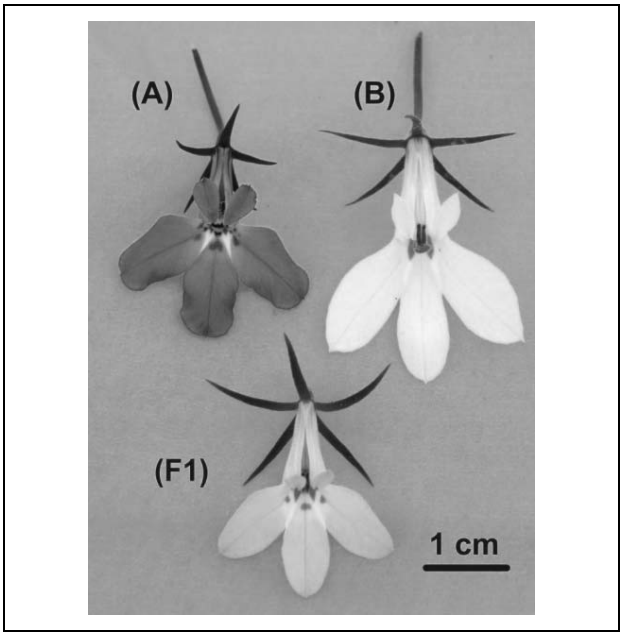


Fig. 5. Flowers of the cross-combination No. 2: *Lobelia erinus* hybrid 'Big Blue' (A) × *Lobelia spec.* polyploid (B) and a typical intermediate type of the F1 progeny (F1).

natural hexaploid with 2n=6x=42 (Lammers and Hensold 1992; Lammers 1993). In contrast, the chromosome counts done in this work revealed a diploid status with 2n=2x=14. There are two possible interpretations. Firstly, the accession used here belongs to another species or, secondly, the accession is a diploid version of the polyploid species *Lobelia cuneifolia*. Only if a clear taxonomical description will be available in the future, it can be decided which of the two possibilities is true.

However, the polyploid form obtained after mutation breeding of the used *Lobelia spec.* used here remained fully fertile despite double polyploidisation from the diploid to the auto-octoploid level. The double polyploidisation can be explained with the repetition of mutagen application. Already mutated cells (2n=4x) might have been affected a second time during a new circle of cell division up to 2n=8x. The maintained fertility expresses a very high generative potential in *Lobelia*. The genus comprises diploid, tetraploid and hexaploid species However, the vigour of this artificial octoploid form, which can be defined by leaf and flower size and compact growth, and its fertility show impressively that the hexaploid level is neither the highest nor the most growth-efficient ploidy level for *Lobelia*. Consequently, superior cultivars which already exist on a hexaploid level for *Lobelia erinus* hybrids (Armand 1912; De Vilmorin and Simonet 1927; Sugiura 1936; Okuno 1937; Thulin 1983) might be upgraded by breeding to the octoploid level, too. Polyploidy and interspecific hybridisation are known as a source of evolution and domestication of ornamental plants (Van Tuyl and Lim 2003; Eeckhaut et al. 2006, Yemets and Blume 2008). As shown by the cross-combination between partners of different ploidy levels, different ploidy levels do not prevent hybridisation in *Lobelia*. For example, the cross-combination of octoploid with tetraploid plants resulted in fertile hexaploid plants. In addi-



Fig. 6. Flower colour changes of the *Lobelia erinus* hybrid 'White Star' from white to blue with rising temperature from left to right (visible in levels of grey).

tion, a heterosis-like effect is visible as shown for the cross-combination *Lobelia spec.* polyploid × *Lobelia erinus* hybrid 'Hot White' regarding flower size (Fig. 4). In future investigations it will be shown whether triploid, pentaploid or heptaploid hybrids can be obtained. If so, it will be worth examining if they already exist among the known cultivars and if they are sterile or possible partly fertile.

Bluish flowering seedlings arose after crossing the pure white *Lobelia spec.* polyploid (octoploid) with the *Lobelia erinus* hybrid 'Hot White' (Fig. 4). This colouration is caused by the synthesis of anthocyanidin under certain environmental conditions like high temperature levels during summer. This colouration phenomenon is a general breeding problem for white flowering vegetatively propagated cultivars of *Lobelia erinus* hybrids like 'White Star' (Fig. 6).

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References

Armand, L. 1912: Fécondation et développement de l'embryon chez les Lobéliacées. Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences **155**, 1534–1536.

Cassells, A.S. and G. Minas 1983: Beneficially infected and chimeral *Pelargonium*: Implication 4 for micropropagation by meristem and explant culture. Acta Hort. **131,** 287–297.

De Vilmorin, R. and M. Simonet 1927: Nombre des chromosomes dans les genres *Lobelia*. Comptes-Rendus Hebdomadaires des Seances et Mémoires de Société de Biologie **96**, 166–168.

Eeckhaut, T., J. Van Huylenbroeck, S. De Schepper and M.C. Van Labeke 2006: Breeding for Polyploidy in Belgian Azalea (*Rhododendron simsii* hybrids). Acta Hort. **714**:113–118.

Hansen, N.J.P. and S.B. Andersen 1996: *In vitro* chromosome doubling potential of colchicine, oryzalin, trifluralin and APM in *Brassica napus* microspore culture. Euphytica **88**, 159–164.

Horn, W. 1996: Zierpflanzenbau. Blackwell Wissenschafts-Verlag, Berlin, Wien, 495–497.

Jadrná, P., O. Plavcová and F. Kobza 2010: Morphological changes in colchicine-treated *Pelargonium* × *hortorum* L.H. Bailey greenhouse plants. Hort. Sci. (Prague) **37**, 27–33.

Lammers, T. and N. Hensold 1992: Chromosome numbers of *Campanulaceae*. II. The *Lobelia tupa* Complex of Chile. Am. J. Bot. **79**, 585–588.

Lammers, T. 1993: Chromosome numbers of *Campanulaceae*. III. Review and Integration of data for subfamily *Lobelioideae*. Am. J. Bot. **80**, 660–675.

Murashige, T. and F. Skoog 1962: A revised medium for rapid growth and bio assays with tobacco tissue cultures. Phys. Plant. **15**, 473–497.

Nilanthi, D., X.L. Chen, F.C. Zhao, Y.S. Yang and H. Wu 2009: induction of tetraploids from petiole explants through colchicine treatments in *Echinacea purpurea* L. J. Biomed. Biotechnol.. Journ. Article ID 343485, 7 pages, doi:10.1155/2009/343485.

Ojiewo, C.O., K. Murakami, P.W. Masinde and S.G. Agong 2007: Polyploidy Breeding in African Nightshade (*Solanum* section *Solanum*). Int. J. Plant Breeding **1**, 10–21.

Okuno, S. 1937: Karyological studies on some species of *Lobelia*. Cytologia, Fuji Jubilee Vol., 897–902.

Olbricht, K., S. Plaschil and F. Pohlheim 2006: Causes of flower colour patterns with focus on chimeral patterns. Floriculture, Ornamental and Plant Biotechnology, Global Science Books, UK. Volume **1**, 311–319.

Plaschil, S., K. Olbricht and F. Pohlheim 2003: Flower colour patterns for ornamental purposes. Acta Hort. **612,** 61–66.

Plaschil, S. and K. Olbricht 2008: Histogenetic variation in flowers of *Angelonia* Humb. et Bonpl. J. Appl. Bot. and Food Quality. **82**, 41–46.

Ruas, P.M., A.L.L. Vanzela, A.O.S. Vieira, C. Bernini and C.F. Ruas 2001: Karyotype studies in Brazilian species of *Lobelia* L., subgenus *Tupa* (*Campanulaceae*). Revta brasil. Bot., Sao Paulo. **24**, 249–254.

Rubuluza, T., R.V. Nikolova, M.T. Smith and K. Hannweg 2007: In vitro induction of tetraploids in *Colophospermum mopane* by colchicines. South Afric. J. Bot., **73**, 259–261.

Satina, S., A.F. Blakeslee and A.G. Avery 1940: Demonstration of the three germ layers in shoot apex of *Datura* by means of induced polyploidy in periclinal chimeras. Am. J. Bot. **27**, 895–905.

Schwanitz, F. 1952: Einige kritische Bemerkungen zur Methode der Bestimmung der Polyploidie durch Messung der Pollen- und Spaltöffnungsgröße. Züchter **22**, 273–275.

Snow, R. 1963: Alcoholic hydrochloric acid-carmine as a stain for chromosomes in squash preparations. Stain Technology **38**, 9–13.

Sugiura, T. 1936: A list of chromosome numbers in angiospermous plants. II. Proceeding of the Imperial Academy of Japan **12**, 144–146.

Thao, N.T.P., K. Ureshino, I. Miyajima, Y. Ozaki and H. Okubo 2003: Induction of tetraploids in ornamental *Alocasia* through colchicine and oryzalin treatments Plant Cell Tiss. Org. **72**, 19–25.

Tilney-Bassett, R.A.E. 1986: Plant Chimeras. Edward Arnold London, Victoria, Baltimore.

Thulin, M. 1970: Chromosome numbers of some vascular plants from East Africa. Botiska Notiser **123**, 488–494.

Thulin, M. 1983: Some tropical African *Lobeliaceae*. Chromosome numbers, new taxa and comments on taxonomy and nomenclature. Nordic J. Botany **3**, 371–382.

Van Tuyl, J.M. and K.B. Lim 2003: Interspecific hybridisation and polyploidisation as tools in ornamental plant breeding. Acta Hort **612**, 13–22.

Viera, A.O.S. and G.J. Shepherd 1996: *Campanulaceae*. IOPB News Letter **26/27**, 24–25.

Wimmer, F.E. 1953: *Campanulaceae-Lobelioidae*. Teil II. In Stubbe, H. and K. Noack (eds.): Das Pflanzenreich Teil 4, Akademie Verlag, Berlin **IV**, 267b, **I–VIII**, 261–813.

Yemets, A.I. and Y.B. Blume 2008: Progress in Plant Polyploidization Based on Antimicrotubular Drugs. The Open Hortic. Journ. **1**, 15–20.

Zander, R. 2002: Handwörterbuch der Pflanzennamen. 17th Edition, Eugen Ulmer, Stuttgart, 538.

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